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Short communication

Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with *Salmonella*

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Abstract

There are many reports of disease due to consumption of cantaloupes contaminated at the surface with enteric pathogens. *Salmonella* is among the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States. Research was undertaken to determine the effects of sanitizer and hot water treatments on microbial populations on cantaloupe surfaces and to determine whether prior decontamination of melons by sanitizer treatment affects vulnerability to recontamination by *Salmonella*. Cantaloupes were sanitized with 200 ppm chlorine or 2.5% hydrogen peroxide solution for 2 min, or hot water (96 °C) for 2 min and were held at 5 °C for 24 h. Hot water treatments reduced the microbial populations on cantaloupe surface by 4.9 log reduction while H_2O_2 or chlorine caused approximately 2.6 log unit reduction on cantaloupe surfaces. When sanitized or hot water treated whole cantaloupes were re-inoculated with *Salmonella*. Higher populations of *Salmonella* were recovered from sanitized cantaloupes than from the untreated controls; recovery was greater from hot water treated cantaloupes than from cantaloupes treated with chlorine or hydrogen peroxide. The results of this study clearly show that sanitized cantaloupes are susceptible to recontamination if exposed to a human bacterial pathogen during subsequent handling.
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Keywords: Cantaloupe; Chlorine; Hydrogen peroxide; Hot water; *Salmonella*

1. Introduction

There are numerous reports of disease due to consumption of fruits and vegetables that were contaminated with enteric pathogens (Beuchat, 1995). Pre- or postharvest contamination most likely originates directly or indirectly from fecal matter. Contributing factors include use of uncomposted manure for fertilizing, irrigation with contaminated water, poor hygiene and unsanitary procedures by field and processing workers, inadequate cleaning and sanitizing of processing equipment, the use of decayed or damaged melons, and failure to wash melons properly prior to packing or fresh-cut processing (Brackett, 1992; Ukuku et al., 2001; Gagliardi et al., 2003). The surface of cantaloupe is covered by a well-developed, shallowly striated, waxy cuticle which varies in thickness but generally conforms closely to cellular outlines (Webster and Craig, 1976). Microstructure of the netting gives the cantaloupe rind inherent surface roughness likely to favor bacterial attachment. The specific source of melon contamination is often unknown. Transfer of *Salmonella* from the cantaloupe rind into the melon flesh by the physical act of cutting the cantaloupe or direct contact with contaminated rinds has been reported (Ukuku and Sapers, 2001). Therefore, the safety of fresh and fresh-cut melons available in salad-bars and

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supermarkets is a concern (Hurst and Schuler, 1992; Tamplin, 1997).

Physical and chemical treatments are used in food processing to eliminate, or at least reduce, the population of pathogenic and spoilage micro-organisms (Wei et al., 1985; Ray, 1992). Washing is one of the very first processing operations to which a fruit or vegetable is subjected. Chlorination of wash water, up to 200 ppm, is routinely applied to reduce microbial contamination in produce processing lines (Wei et al., 1995). However, the use of chlorine is of concern due to the potential formation of harmful by-products (Richardson et al., 1998) and typically can only achieve a 1–2 log reduction of native microflora (Ukuku et al., 2001). In this study, we investigated sites of bacterial attachment on surface structures of cantaloupe rind; the effects of chlorine, hydrogen peroxide and hot water treatments on the cantaloupe surface and on populations of native microflora and *Escherichia coli* population on inoculated cantaloupes; and the effects of sanitizer treatments on *Salmonella* recontamination of cantaloupe.

2. Materials and methods

2.1. Bacterial strains, growth conditions, and preparation

Bacterial strains used in this study were *Salmonella* Stanley H0558, *Salmonella* Newport H1275, *Salmonella* Anatum F4317, *Salmonella* Infantis F4319 (all associated with alfalfa sprout-related outbreaks, obtained from Dr. Patricia Griffin, Center for Disease Control (CDC, Atlanta); and *Salmonella* Poona RM2350 (associated with a cantaloupe-related outbreak, obtained from Dr. Robert Mandrell, ARS, USDA). Bacteria were maintained on Brain–Heart Infusion Agar (BHIA, BBL/Difco, Sparks, MD) slants held at 4 °C. Prior to use, the cultures were subjected to two successive transfers by loop inocula to 5 ml Brain–Heart Infusion Broth (BHIB, BBL/Difco). A final transfer of 0.2 ml was made into 20 ml BHIB with incubation at 36 °C for 18 h under static conditions. The bacterial cells were harvested by centrifugation (10,000*g*, 10 min) at 4 °C. The cell pellets were washed in salt-peptone [0.85% NaCl, 0.05% Bacto-peptone (BBL/Difco)]. The cell pellets were used to prepare three different types of inoculum as stated below. The first inoculum type consisted of an inoculum cocktail containing all five *Salmonella* serovars at $\sim 10^2$ CFU/ml. The second inoculum type consisted of an inoculum cocktail containing all five *Salmonella* serovars at $\sim 10^4$ CFU/ml. The third inoculum type consisted of an inoculum cocktail containing all five *Salmonella* serovars at $\sim 10^6$ CFU/ml. All three inocula were prepared in 3 l of 0.1% (w/v) peptone-water (PW).

2.2. Sanitizer treatments

Four treatments were compared: sterile tap water, 200 ppm chlorine, 2.5% hydrogen peroxide, and hot water (96 °C). The 200 ppm chlorine solution was prepared by diluting Clorox® commercial bleach containing 5.25% NaOCl in sterile tap water and adjusting the pH to 6.4 ± 0.1 by adding citric acid (Mallinckrodt, Paris, KY). Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the US Environmental Protection Agency. A 2.5% hydrogen peroxide solution was prepared from a 30% stock solution (Fisher Scientific, Suwanee, GA) by dilution with sterile tap water. Cantaloupes ("Western shippers") purchased from a local supermarket were allowed to come to room temperature (~25 °C) before being sanitized. All cantaloupes were treated as follows: unwashed (control); washed with water, 200 ppm chlorine or 2.5% hydrogen peroxide solutions for 2 min; or submerged in hot water (96 °C) for 2 min. The concentrations of sodium hypochlorite and hydrogen peroxide or the hot water (96 °C) treatments were chosen based on the results of our previous work (Sapers and Simmons, 1998; Ukuku et al., 2004a, b). All washing treatments were performed by constant agitation with a glove-covered hand. Cantaloupes were treated individually in 3 l of hot water (96 °C) or freshly prepared sanitizer solution, and treated cantaloupes were then placed inside a biosafety cabinet at ambient temperature to dry for 1 h before exposure to *Salmonella* contaminant.

2.3. Inoculation of cantaloupes

Sanitized cantaloupes stored at 5 °C for 7 days were randomly selected immediately, 3 and 7 days after sanitizing application, and were inoculated with different level of *Salmonella* populations. Cantaloupes were submerged in 3 l of bacterial inoculum and agitated by stirring with a glove-covered hand for 10 min to ensure uniform inoculation. After inoculation, the cantaloupes were placed inside a biosafety cabinet to dry for 1 h. Unsanitized or untreated cantaloupes were subjected to the same treatments and then were analysed for level of contamination with *Salmonella*.

This study was designed to investigate whether differences exist in the extent of attachment of *Salmonella* when sanitized cantaloupes are exposed to the contaminant immediately following sanitizing or up to 7 days later, as might occur in the packinghouse or supermarket, respectively. Two cantaloupes per treatment were sampled in each of three separate experiments.

2.4. Enumeration of attached bacterial cells

To enumerate attached, viable *Samonella*, cantaloupe surfaces were randomly cut with a sterilized stainless

steel cork-borer to produce rind plugs of 22 mm in diameter with an external rind surface area (πr^2) of 3.80 cm^2. A total of about 150 rind plugs per cantaloupe were obtained. The interior flesh adhering to the rind plugs was trimmed off using a sterilized stainless-steel knife. Cantaloupe rind plugs (70,~25 g) were blended (Waring commercial blender, speed set at level 5 for 1 min) with 75 ml of 0.1% PW and decimal dilutions of the samples were made with 0.1% PW. Plate count agar (PCA) with incubation at 30 °C for 72 h was used for enumeration of mesophilic aerobic bacteria. *Pseudomonas* spp. were enumerated by plating 0.1 ml on *Pseudomonas* isolation agar (Difco) with incubation at 27 °C for 3 days. MRS + 0.08% sorbic acid with an overlay of the same media in an anaerobic chamber with incubation at 30 °C for 48 h was used for lactic acid bacteria. Czapek Malt Agar (CMA) with incubation at 30 °C for 72 h was used for yeast and mold. For the *Salmonella* study, decimal dilutions of the sample were made with 0.1% PW, and aliquots (0.1 ml) were plated in duplicate on XLT4 Agar (BBL/Difco). For comparison, a pure culture of *Salmonella* Poona was plated on XLT4, incubated as above, and run in parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the FDA Bacteriological Analytical Manual following conventional biochemical methods (Andrews et al., 1995).

2.5. Data analysis

All experiments were done in triplicate with duplicate samples analysed at each sampling time. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, NC) for analysis of variance (ANOVA). Mean values of bacterial cell numbers on treated and untreated cantaloupe were compared to determine significant differences at ($p < 0.05$) using the Bonferroni LSD method (Miller, 1981).

3. Results and discussion

3.1. Effect of sanitizer/hot water treatments on bacterial populations of cantaloupe surface

Washing inoculated cantaloupe with water (20 °C) did not significantly reduce the native microflora of cantaloupe surfaces (Table 1). Hot water treatments were very effective in reducing the population of native microflora and the bacterial populations was significantly ($p < 0.05$) reduced than the populations on chlorine or hydrogen peroxide treated cantaloupes (Table 1). Although, chlorine or hydrogen peroxide treatments were less effective than hot water treatments but there had smaller microbial populations compared to control and water washed cantaloupes. In this experiment, the population of *Pseudomonas* spp. or lactic acid bacteria and yeasts and molds on cantaloupe surfaces that survived chlorine or hydrogen peroxide treatment averaged 1.8 and < 0.7 log cfu/cm^2, respectively, while the populations of *Pseudomonas* spp. or lactic acid bacteria and yeasts and molds that survived treatment with hot water averaged < 0.8 log cfu/cm^2 (Table 2). There were a significant difference in population reduction for *Pseudomonas* spp., lactic acid bacteria and yeasts and molds on cantaloupe surfaces treated with hot water than those sanitized immediately after purchase.

Table 1
Population of total bacterial counts from cantaloupe rind surface treated with sanitizers and hot water during storage at 5 °C for up to 7 days

Treatment	Day 0	Day 3	Day 7
Control	6.9 ± 0.2 A	6.2 ± 0.4 A	6.8 ± 0.3 A
Water washed	6.6 ± 0.3 A	6.9 ± 0.1 A	7.1 ± 0.3 A
Chlorine (200 ppm)	4.2 ± 0.2 A	3.6 ± 0.3 B	3.5 ± 0.3 B
Hydrogen peroxide (2.5%)	4.3 ± 0.2 A	3.9 ± 0.4 A	3.6 ± 0.2 B
Water (96 °C)	2.3 ± 0.2 A	2.0 ± 0.1 A	2.3 ± 0.1 A

Mean data in each row not followed by the same letter are significantly different ($p < 0.05$).

3.2. Effect of sanitizer and hot water treatment on subsequent attachment of Salmonella to cantaloupe rind

Salmonella was not isolated from the surface of the cantaloupes purchased from the distributor prior to inoculation. The populations of *Salmonella* recovered from the surface of inoculated untreated or sanitized cantaloupes immediately after treatments are shown in Table 3. Sanitizer and hot water treatment of cantaloupe surfaces which reduce the native microflora (Table 1) allowed for increased attachment of the pathogen. The population of *Salmonella* attached on the surface of cantaloupe treated with hot water was significantly ($p < 0.05$) higher than for the untreated controls or for cantaloupes sanitized with chlorine or hydrogen peroxide. The populations of *Salmonella* recovered from the surfaces of control or sanitized cantaloupe 3 or 7 days after inoculation were not significantly ($p < 0.05$) different from melons inoculated immediately following sanitizing treatments. However, an average of 0.8 and 1 log increase was recovered at day 7 on cantaloupe surfaces sanitized with chlorine or treated with hot water, respectively. At day 3, the average population recovered on the same type of melon increased by 0.2 log.

Ukuku et al. (2001) reported variability in total plate counts of surface microflora of cantaloupes and attributed this observation to cantaloupe surface irregularities such as roughness, crevices, and pits.

Table 2
Surviving population of spoilage microflora on cantaloupe rind surface treated with sanitizers and hot water

Survivors (log CFU/cm^2)					
Treatment	Control	Water-washed	H_2O (96 C)	Cl_2 (200 ppm)	H_2O_2 (2.5%)
Pseudomonas spp.	2.14±0.13 A	2.16±0.10 A	0.6±0.2 C	1.76±0.11 B	1.76±0.11 B
Yeast and mold	2.8±0.1 A	2.5±0.1 A	0.1±0.0 B	0.1±0.1 B	0.1±0.0 B
Lactic acid bacteria	3.6±0.2 A	3.4±0.2 A	0.8±0.2 D	1.8±0.2 C	2.1±0.2 B

Mean data in each row not followed by the same letter are significantly different ($p<0.05$).

Table 3
Re-contamination of sanitized cantaloupe surface with *Salmonella* populations at 0, 3 or 7 days after sanitizer treatments[a]

Salmonella on cantaloupe rind (log CFU/cm^2)[b]			
Treatment	Day 0	Day 3	Day 7
Control	3.5±0.2 C	3.7±0.1 C	3.7±0.1 D
Water	3.6±0.2 C	3.7±0.1C	3.3±0.3 D
Cl_2	4.6±0.1 B	4.9±0.1 B	5.4±0.3 B
H_2O_2	4.2±0.1 B	4.5±0.1 B	4.4±0.2 C
H_2O (96 °C)	5.4±0.1 A	5.7±0.2 A	6.4±0.4 A

[a]Initial inoculum of *Salmonella* used was 10^8 CFU/ml.
[b]Mean data in each column not followed by the same letter are significantly different ($p<0.05$).

Previously we reported that hot water decontamination of cantaloupes designated for fresh-cut processing could have major advantages over the use of sanitizers, including a significant reduction or elimination of *Salmonella* on melon surfaces and reduced transfer of this pathogen from the rind to the flesh during fresh-cut processing (Ukuku et al., 2004a, b). Our current study indicates that hot water treatments greatly reduced the native microflora and should reduce the probability of transfer of these bacteria from the rind to the flesh during cutting. Although, chlorine or hydrogen peroxide treatments significantly reduced populations of bacteria attached on the cantaloupe surface, the level of decontamination achieved, would not have been sufficient to assure microbiological safety. Other studies on efficacy of chlorine treatment of fresh produce reported incomplete removal or inactivation of bacteria (Beuchat, 1995; Brackett, 1992). Submerging the melons in hot water appears to be a better antimicrobial treatment than washing with chlorine or hydrogen peroxide. Annous et al. (2004) demonstrated that hot water surface pasteurization of cantaloupes with pilot-scale equipment enhanced microbiological safety and extended shelf life. Cantaloupe rind is thick and dense, and if heat exposure is not excessive, the internal flesh used for fresh-cut processing will be unaffected. Thus, hot water surface pasteurization provides a means of reducing the risk of enteric disease through consumption of contaminated fresh or fresh-cut melons.

However, the possibility of human pathogen contamination after melons are sanitized through cross-contamination or improper handling during distribution or in the supermarket must be considered. Such re-contamination of cantaloupes could result from inadequate sanitation of conveyors and other equipment in the packinghouse downstream of the sanitizing operation; poor hygiene by packinghouse workers or supermarket employees; or cross-contamination in the supermarket during unpacking, display, in-store fresh-cut processing or handling by shoppers. The increased attachment of *Salmonella* to whole melons treated with sanitizers or hot water (96 °C) could be attributed to the presence of a finite number of microbial binding sites, some of which are made available by the sanitizer treatments, or to chemical and/or physical changes in the melon surface that are imparted by the treatment (Ukuku et al., 2004a, b). Our results indicate that exposure of chemically or hot water sanitized cantaloupes to *Salmonella* could lead to higher levels of attachment than would occur if the melons had not been sanitized. This suggests the need for great care to avoid recontamination of sanitized melons in the packinghouse and during subsequent distribution and marketing.

In conclusion, the results of this study showed differences in effects of chemical and hot water sanitizer treatments in reducing the load of attached bacteria on cantaloupe surfaces. Plate count data quantify the superiority of hot water treatment over chlorine and hydrogen peroxide in reducing populations of native microflora. Hot water surface pasteurization of cantaloupes prior to fresh-cut preparation is a viable food safety option. However, if such melons are not processed immediately and are exposed to human pathogen contaminants such as *Salmonella*, the sanitized melons are easily re-contaminated, presumably due to the reduced populations of native microflora.

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